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and elicited macrophages in suspension or while adherent [3], it has been suggested that the state of macrophage activation may influence membrane protein movement [2]. Our present study in which adherent, resident macrophages and suspension exudate macrophages did not cap suggests that under certain conditions (i.e., pharmacologic manipulation) differences in macrophage membrane protein movement may become evident.

The ability to cap is not related to Ia expression as our populations contained less than 5% Ia-positive cells. It is probable that some, as of yet, not recognized macrophage heterogeneity is responsible for this phenomenon. The number of caps did not increase in cells treated with colchicine, a drug known to enhance some types of cap formation but not others [12]. Cytochalasin D inhibited the cap formation, suggesting an intact contractile apparatus is necessary for cap formation (B. Woda, unpublished observation). The mechanism by which capping occurs in these cells remains to be determined.

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Antagonists of chemoattractants reveal separate receptors for cAMP, folic acid and pterin in *Dictyostelium*

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Summary. Adenosine 3',5'-monophosphate (cAMP), folic acid and pterin are chemoattractants in the cellular slime molds. The cAMP analog, 3'-amino-cAMP, inhibits a chemotactic reaction to cAMP at a concentration at which the analog is chemotactically inactive. The antagonistic effect of 3'-amino-cAMP on the chemotactic activity of cAMP is competitive, which suggests that 3'-amino-cAMP antagonizes cAMP via the chemotactic receptor for cAMP. 3'-Amino-cAMP does not antagonize folic acid or pterin. The binding of folic acid to post-vegetative *Dictyostelium discoideum* cells is inhibited by low concentrations of 2-deamino-2-hydro folic acid (DAFA [7]). DAFA is neither chemotactically active, nor does it inhibit a chemotactic reaction to folic acid. This questions the involvement of the main folic acid cell surface-binding sites in the chemotactic response to folic acid. The pterin analog, 6-aminopterin, is an antagonist of pterin, but not of cAMP or folic acid. Our results show that cAMP, folic acid and pterin are detected by different receptors. Furthermore, they suggest that the antagonistic action of 3'-amino-cAMP and 6-aminopterin is localized in the signal transduction pathway at a step before the signals from the separate receptors have arrived at a single pathway.

cAMP acts as a chemoattractant in aggregative *D. discoideum* cells [1], and in three other related species [2]. In the post-vegetative phase the threshold concentration of cAMP for a chemotactic response is 10^{-6} – 10^{-7} M in these species ([3], and unpublished observations). Post-vegetative cells

also react chemotactically to folic acid [4], and to pterin [5].

Recently we have observed that several cAMP derivatives act as antagonists of cAMP [6]. These compounds inhibit a chemotactic reaction to cAMP at a concentration at which they are chemotactically inactive. One of these derivatives is 3'-amino-cAMP.

cAMP, folic acid and pterin are probably detected by cell surface receptors. The binding of folic acid to post-vegetative *D. discoideum* cells is inhibited by low concentrations of 2-deamino-2-hydroxy folic acid (DAFA) [7]. Since DAFA is chemotactically inactive [8], this would suggest that DAFA is an antagonist of folic acid.

In *D. lacteum* 6-aminopterin is at least 1000-fold less active than pterin, which is unexpected, since most 6-substituted pterins have approximately the same chemotactic activity as pterin (unpublished observations). The low chemotactic activity of 6-aminopterin could mean that it acts as an antagonist.

We have investigated the chemotactic activity of cAMP, folic acid and pterin in the absence and presence of 3'-amino-cAMP, DAFA, and 6-aminopterin in the post-vegetative phase of four species of *Dictyostelium*. The results indicate that only 3'-amino-cAMP and 6-aminopterin function as antagonists. No antagonistic effect was observed with DAFA.

Methods

D. discoideum (NC-4 H) was grown in association with *Escherichia coli* B/r on SM-agar [9], and *D. purpureum*, *D. lacteum*, and *D. minutum* (V₈) on a 0.1% lactose-peptone agar. Cells were harvested, washed and plated [9]; chemotaxis was tested with the small population assay [10], as described previously [6]. Folic acid deaminase and cAMP phosphodiesterase activity were isolated from *D. discoideum* by starvation of cells in 10 mM phosphate buffer pH 6.0 at a density of 10^7 cells/ml. After 3 h the cells were centrifuged and the supernatant was used to convert folic acid into

DAFA. DAFA was purified by high performance liquid chromatography (HPLC) and characterized by its UV spectrum [11], electrophoretic mobility at pH 6.0, and by the quantitative release of glutamic acid by acid hydrolysis. 6-Aminopterin was synthesized and purified as described [12]. The compound was further purified by HPLC and characterized by its UV spectrum [13]. 3'-Amino-cAMP was a generous gift by Dr Jastorff.

Deamination of 6-aminopterin, pterin and folic acid was analysed by HPLC on a cation exchanger at pH 2.0 and hydrolysis of 3'-amino-cAMP and cAMP was analysed by HPLC on an anion exchanger at pH 5.3.

Results

Post-vegetative *D. discoideum* cells (starved for 1 h) do not react chemotactically to 3'-amino-cAMP (table 1). Addition of 10^{-3} M 3'-amino-cAMP to various concentrations of cAMP reduces the chemotactic activity of cAMP about 100-fold. Apparently, 3'-amino-cAMP is an antagonist of cAMP.

3'-Amino-cAMP antagonizes only cAMP, 6-aminopterin antagonizes only pterin, and none of the additives antagonizes folic acid (table 1). Similar results were found with *D. purpureum*, except that 6-aminopterin is slightly more active (less than 3-fold) in this species than in *D. discoideum*, and that 6-aminopterin reduces the chemotactic activity of pterin about 10-fold. *D. lacteum* cells do not react to cAMP, but are specifically sensitive to pterin. 6-Aminopterin is chemotactically inactive, and an antagonist of pterin. DAFA has a chemotactic activity at high concentrations, but it has no antagonizing effect on the activity of folic acid. *D. minutum* cells are very sensitive to folic acid, and DAFA has the same chemotactic activity as folic acid in this species [14]. Pterin and 6-aminopterin have similar chemotactic activities in this species.

Is the antagonistic activity derived from an interaction of the antagonist with the chemoreceptor? The results of fig. 1 show that the antagonistic effect of 3'-amino-

Table 1. Threshold concentrations^a of chemoattractants, antagonists of chemoattractants, and mixtures of them

Test substance	Additive	<i>D. discoideum</i>	<i>D. purpureum</i>	<i>D. lacteum</i>	<i>D. minutum</i>
cAMP	—	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁶ –10 ⁻⁷ M	>10 ⁻³ M	>10 ⁻³ M
cAMP	3'-NH-cAMP 10 ⁻³ M	10 ⁻⁴ –10 ⁻⁵ M	10 ⁻⁴ –10 ⁻⁵ M	nd (1)	nd (1)
cAMP	DAFA 10 ⁻⁴ M	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁶ –10 ⁻⁷ M	nd (1)	nd (1, 2)
cAMP	6AP 10 ⁻⁴ M	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁶ –10 ⁻⁷ M	nd (1)	nd (1, 2)
FA	—	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁵ –10 ⁻⁶ M	10 ⁻⁸ –10 ⁻⁹ M
FA	3'-NH-cAMP 10 ⁻³ M	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁵ –10 ⁻⁶ M	10 ⁻⁸ –10 ⁻⁹ M
FA	DAFA 10 ⁻⁴ M	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁵ –10 ⁻⁶ M	nd (2)
FA	6AP 10 ⁻⁴ M	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁵ –10 ⁻⁶ M	nd (2)
Pterine	—	10 ⁻⁵ –10 ⁻⁶ M	10 ⁻⁵ –10 ⁻⁶ M	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁵ –10 ⁻⁶ M
Pterine	3'-NH-cAMP 10 ⁻³ M	10 ⁻⁵ –10 ⁻⁶ M	10 ⁻⁵ –10 ⁻⁶ M	10 ⁻⁶ –10 ⁻⁷ M	10 ⁻⁵ –10 ⁻⁶ M
Pterine	DAFA 10 ⁻⁴ M	10 ⁻⁵ –10 ⁻⁶ M	10 ⁻⁵ –10 ⁻⁶ M	10 ⁻⁶ –10 ⁻⁷ M	nd (2)
Pterine	6AP 10 ⁻⁴ M	10 ⁻³ –10 ⁻⁴ M	10 ⁻⁴ –10 ⁻⁵ M	10 ⁻⁴ –10 ⁻⁵ M	nd (2)
3'-NH-cAMP	—	>10 ⁻³ M	>10 ⁻³ M	>10 ⁻³ M	>10 ⁻³ M
DAFA	—	>10 ⁻³ M	>10 ⁻³ M	10 ⁻³ –10 ⁻⁴ M	10 ⁻⁸ –10 ⁻⁹ M
6AP	—	10 ⁻³ –10 ⁻⁴ M	10 ⁻³ –10 ⁻⁴ M	>10 ⁻³ M	10 ⁻⁵ –10 ⁻⁶ M

^a The threshold concentrations of the test substances for a chemotactic reaction were measured with the small population assay after cells had been on the test plates for 1 h. The threshold is given by two concentrations: less than 50% of the populations reacted positively at the lower concentration, and more than 50% reacted positively at the higher concentration. The highest concentration used was 10⁻³ M.

nd (1), Not determined, because the test substance was inactive.

nd (2), Not determined, because the additive had chemotactic activity.

FA, Folic acid; 3'-NH-cAMP, 3'-amino-cAMP; 6AP, 6-aminopterin.

cAMP is competitive [15], which indicates that the action of 3'-amino-cAMP is at or after the chemotactic receptor for cAMP.

Is the rate of degradation of the antagonists involved in their action? 6-Aminopterin is deaminated by *D. discoideum* about three times slower than pterin. The product, 6-aminolumazine, is chemotactically inactive, and has lost the antagonistic properties (data not shown). 3'-Amino-cAMP is not hydrolysed by *D. discoideum* phosphodiesterase, but the compound is hydrolysed by phosphodiesterase from beef heart (Boehringer) at about 1–3 times lower rates than cAMP (Dijkgraaf & Van Haastert, unpublished observations). In order to reveal the involvement of degradation of 3'-amino-cAMP in its antagonistic action we added various amounts of beef heart phosphodiesterase to suspensions of post-vegetative *D. discoideum* cells. The activity of

beef heart phosphodiesterase was up to 100 times higher than the phosphodiesterase activity of post-vegetative *D. discoideum* cells. Beef heart phosphodiesterase is stable in a suspension of *D. discoideum* cells for at least 2 h. The cell suspensions with various beef heart phosphodiesterase activities were used in the chemotactic assay. The threshold activity of cAMP remained 10⁻⁶–10⁻⁷ M; 3'-amino-cAMP remained chemotactically inactive, and its antagonistic effect on cAMP was still the same as shown in table 1. We therefore conclude that degradation of 3'-amino-cAMP or 6-aminopterin is not involved in the antagonistic effects of these compounds.

Discussion

DAFA is chemotactically inactive in *D. discoideum* cells, and does not inhibit the chemotactic activity of folic acid. Since

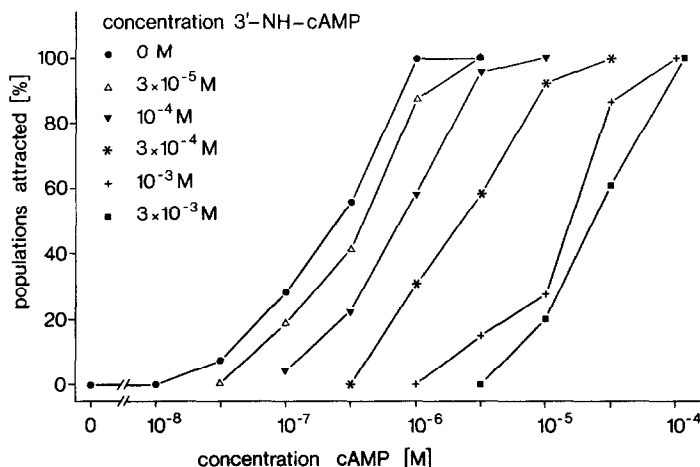


Fig. 1. The chemotactic activity of various mixtures of cAMP and 3'-amino-cAMP in *D. purpureum*.

DAFA competes with folic acid for binding sites on the cell surface of *D. discoideum* [7], it seems unlikely that these binding sites are the chemotactic receptors for folic acid. It is not known whether other folic acid-binding sites which do not bind DAFA are present on the cell surface of *D. discoideum*.

Table 1 shows that 6-aminopterin has only antagonizing effects on pterin and not on folic acid or cAMP, and 3'-amino-cAMP antagonizes cAMP, but not folic acid or pterin. Furthermore, we have shown (fig. 1) that 3'-amino-cAMP antagonizes cAMP via the chemotactic receptor of cAMP. These results indicate that cAMP, folic acid and pterin are detected by different receptors. Additionally, the results show that the action of the antagonists is located in the signal transduction pathway at a step at or after the receptor, but before the signals from cAMP, folic acid and pterin receptors have been focused into a single pathway. Further studies on the exact location of the action of the antagonists and on the mechanism of their action may be helpful for the elucidation of the transduction of chemotactic signals in *D. discoideum*.

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